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A DIVISION OF QUANTA BIODESIGN LIMITED

64-0614-0900

Q-Bright® Extreme Chemiluminescent Western Blot Kit

64-0414-0900

Q-Bright® Extreme chemiluminescent detection kit is a high sensitivity, luminol-based substrate for horseradish peroxidase (HRP), providing a high sensitivity signal. Q-Bright® Extreme provides much greater sensitivity compared to Q-Bright® Endure and is comparable to many of the available commercial reagents providing mid to low-femtogram levels of detection. The Western Blot Kit includes western blot wash buffer (10X TBS + Tween® 20), Q-Bright® Western Blot Blocking Buffer (TBS), Anti-Mouse and Anti-Rabbit HRP conjugated secondary antibodies, and Q-Bright® Extreme Substrate and Stable Peroxide. For best results, use the recommended antibody concentrations for each Q-Bright® product.

Primary Antibody	Secondary Antibody	Q-Bright® Extreme Sensitivity Range
10-200ng/ml *	1-20ng/ml*	Mid to low-Femtogram

* Optimal antibody concentrations determined by end user.

Kit Contents

Component Name	PN	Quantity	Concentration
10X Tris Buffered Saline with Tween® 20 (10X TBS-T)	41-0200-0400	25ml	Final 1X Concentration: 25mM Tris, 150mM Sodium Chloride, 0.05% Tween® 20
Q-Bright® Western Blocking Buffer (TBS)	43-0400-0600	100ml	RTU
Goat anti-Mouse IgG (H&L), Horseradish Peroxidase min X Rabbit, Human, Bovine, Horse	11-0101-0303	50ul	1mg/ml
Goat anti-Rabbit IgG (H&L), Horseradish Peroxidase min X Mouse, Human, Bovine, Horse	11-0201-0503	50ul	1mg/ml
Q-Bright® Plus Extreme Substrate	32-0209-0200	10ml	RTU
Q-Bright® Plus Extreme Peroxide	32-1222-0200	10ml	RTU

Store Kit at 4ºC. Kit components may contain 0.1% Kathon as a preservative.
The TBS-T may form precipitates at 4ºC. Bring to room temperature and gently mix to dissolve before use.
Protect Horseradish peroxidase conjugated secondary antibodies and Q-Bright® reagents from light exposure.

Additional Materials Required

- Protein samples separated by gel electrophoresis and transferred to a PVDF or nitrocellulose membrane following manufacturer's instructions.
- Primary antibody from a murine or rabbit host
- 1X Tris buffered saline (optional)
- CCD Imager or x-ray developing system

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Western Blot Assay Overview

- Separate proteins by gel electrophoresis
- Transfer proteins from gel to the membrane
- Block non-specific binding sites on the membrane
- Incubate in Primary antibody
    - Primary antibody binds to protein of interest
- Wash to remove excess Primary antibody
- Incubate in HRP-conjugated Secondary antibody
    - Secondary antibody binds to Primary antibody
- Wash to remove excess antibodies
- Apply Q-Bright® Chemiluminescent Substrate
    - Q-Bright® reacts with HRP to generate a luminescent signal
- Image

Procedure

1. Prepare 1X Wash Buffer (TBS-T) by combining 25ml of 10X stock with 225ml deionized water.
2. Block membrane at room temperature for 30-60 minutes in Q-Bright® Blocking Buffer (TBS) with gentle agitation. Ensure the volume is sufficient to fully cover the membrane (approximately 10ml per mini blot).
3. Wash once with 1X Wash Buffer (TBS-T). Ensure the volume is sufficient to fully cover the membrane (approximately 10ml per mini blot).
4. Dilute primary antibody 10-200ng/ml in Q-Bright® Blocking Buffer (TBS) and fully cover membrane. Incubate for 1.5 hours at room temperature or in 4°C overnight with gentle agitation

    Optimal concentration of primary antibody must be determined by end user.

    Tween® 20 can be added to the primary antibody incubation step.
5. Wash membrane 3 times for 5 minutes each with 1X Wash Buffer (TBS-T)
6. Dilute AQ secondary antibody to 1-20ng/ml in 10ml of blocking buffer and incubate for 60 min at room temperature with gentle agitation.

    Use the Goat anti-Rabbit, HRP conjugate with rabbit host primary antibodies or the Goat ant-Mouse, HRP conjugate with murine host primary antibodies.

    Optimal concentration of secondary antibody can be optimized by the end user.

    Tween® 20 can be added to the secondary antibody incubation step.
7. Wash membrane 3 times for 5 minutes each with 1X Wash Buffer (TBS-T).

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To reduce background, the membrane can be washed one time with TBS (without Tween® 20; not provided) before adding the Q-Bright® working solution.

8. Prepare Q-Bright® Extreme working solution by combining equal parts of luminol enhancer solution and stable peroxide. Use 0.1ml of working solution per cm² of membrane. For best results equilibrate working solution to room temperature before use and protect from light.
9. Incubate membrane in Q-Bright® Extreme working solution for 5 minutes with gentle agitation. Allow excess substrate to drip off the membrane and transfer to transparent plastic wrap. Gently smooth out any air bubbles and image with a CCD imaging system or x-ray film.

Q-Bright® Quick Start Guide				
	Enhance Plus	**Endure**	**Endure Plus**	**Extreme**
Lower limits of detection	~Low Picogram	~High to Mid Femtogram	~Mid Femtogram	~Mid to low femtogram
Primary Antibody	0.2-10ug/ml	0.02-1ug/ml	0.02-0.5ug/ml	10-200ng/ml
Secondary Antibody - HRP Conjugate	20-100ng/ml	4-20ng/ml	2-20ng/ml	1-20ng/ml
Comparable Reagents	SuperSignal® Pico Plus	SuperSignal® West Dura		SuperSignal® West Femto
	Robust signal for routine and high abundance proteins	Long lasting, stable signal for abundant proteins	High sensitivity with limited optimization needed.	Highest sensitivity, requires optimization for maximum signal to noise.

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Troubleshooting Tips

Issue	Cause	Solution
High background	• High antibody concentration • Unclean equipment	• Reduce primary or secondary antibody concentration • Clean western trays, tweezers, and any equipment that contacts the membrane with 70% ethanol before use
Weak bands or signal	• Not enough antibody or antigen in the system • Substrate is not sensitive enough for detection	• Optimize antibody dilution • Load more protein
Non-specific bands	• Primary antibody concentration too high	• Reduce Primary antibody-optimize system • Use a secondary antibody that has been adsorbed against target tissue source

	• Inadequate antibody specificity • Reactivity of secondary antibody to tissue source	